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Embryogenesis in Hydra

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Abstract. Embryogenesis in hydra includes a variable period of dormancy; and this period, as well as subsequent stages through hatching, takes place within a thick cuticle that hinders observation. Thus, although the early stages of development have been well-characterized qualitatively, the middle and later stages are only poorly understood. Here, we provide a detailed description of the stages of embryogenesis, including the time required to traverse each of the stages, and the changes that occur in the type and number of cells throughout the stages. The events of cleavage and gastrulation occur within the first 48 h. Cleavage is holoblastic and unipolar and leads to a single-layered coeloblastula. Gastrulation occurs by ingression and is followed by the deposition of the thick cuticle. Thereafter, during the variable period of dormancy ranging from 2–24 weeks, little occurs; the important events are the conversion of the outer layer into an ectoderm and the appearance of the interstitial cell lineage. During the last 2 days before hatching, the endoderm and gastric cavity form, while stem cells of the interstitial cell lineage proliferate and differentiate into neurons, nematocytes, and secretory cells. Finally, the cuticle cracks, and the hatchling enlarges and emerges from the cuticle as a functional animal. The formation of the gastric cavity and the hatching of the embryo are both explicable in terms of the osmotic behavior of the animal and the hydrostatic forces generated by this behavior. Characteristics of development that are common to hydra and triploblastic phyla are presented.

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Introduction

Hydra, like all cnidarians, arose very early in evolution. Its diploblastic body plan is therefore simple, as is its embryogenesis. A comparison of this simple embryogenesis with those of the more complex triploblastic metazoans would reveal those features that are common to all animals. And a further investigation of those common features should then uncover basic genetic mechanisms underlying development. The Hox genes, for example, play an important role in specifying morphological regions in insects and vertebrates—and these genes also occur in cnidarians (Schierwater *et al.*, 1991; Murtha *et al.*, 1991; Schummer *et al.*, 1992); *Cnox-2* is a Hox gene that is involved in maintaining the adult pattern of hydra (Shenk *et al.*, 1993a, b). Identifying these regulatory genes and understanding their roles in development will depend on a detailed knowledge and understanding of hydra embryogenesis.

The epithelial cells in the adult hydra are constantly proliferating, changing their relative position along the body axis, and differentiating. Thus the patterning processes that maintain the form of the animal are continuously active in the adult as well as the embryo (Campbell, 1967a, b; Otto and Campbell, 1977). The effects of the patterning processes on cells and tissues are well understood, and current efforts are focused on understanding their molecular bases. The processes that set up the pattern in the embryo could be the same, overlap with, or differ from those maintaining pattern in the adult. Resolving this uncertainty in hydra will require a precise description of embryogenesis.

After more than a century of study, the early stages of hydra embryogenesis—cleavage through cuticle

formation—have been well described qualitatively (Kleinenberg, 1872; Brauer, 1891; Tannreuther, 1908; Kanev, 1952; Tardent, 1966). But the stages have not been timed, and neither the rates of cell division nor the number of cells in each stage have been measured. In contrast to the early stages of embryogenesis, the later ones are poorly understood; a single report (Brauer, 1891) provides a rough outline of the events, but detail is lacking. This lack of information is due to four factors. First, many strains of hydra species do not produce gametes under culture conditions in the laboratory. Second, most cultured strains and species also have low hatching rates. Third, the early and late stages of embryogenesis are separated by a period of dormancy that is extremely variable (2–52 weeks). Finally, a thick cuticle covers the embryo through most of embryogenesis; thus development is difficult to examine during the dormant period or during the final stages leading to hatching.

We have taken advantage of two strains, one female and one male, that produce gametes continuously in the laboratory. Although the hatching time remains quite variable, the number of embryos produced is high and the hatching rate is close to 100%. We could therefore describe the stages of development in detail; we could also time them and quantify the cell populations during embryogenesis.

Materials and Methods

Culture of adult hydra and embryos

Most analyses were carried out with two strains. One was PA1, a female strain of *Hydra vulgaris*, isolated from a pond on the Haverford College campus near Philadelphia, Pennsylvania, by Dr. Carolyn Teragawa. The other, CA7, a male strain of *Hydra vulgaris*, was isolated by Drs. Lynne Littlefield and Carolyn Teragawa at Boulder Creek, near Susanville, California. Both strains were maintained in the laboratory for 3 years before the current studies were undertaken. Some analyses were carried out with the male E2 and female A5 strains, which were derived from crosses of PA1 and CA7. All strains were cultured in hydra medium, which consisted of a commercial spring water (Arrowhead) supplemented with 1 m*M* $CaCl_2$. Animals were maintained at 15°C with a light/dark regime of 12 hours light and 12 hours dark; they were fed once a week with the nauplii of the brine shrimp, *Artemia salinas* (San Francisco Brand), and the medium was changed three times per week. Under these culture conditions, the males produced sperm most of the time. Individual females produced one-to-several eggs at roughly monthly intervals, and often a culture of females produced eggs simultaneously.

To obtain fertilized eggs, six male hydra bearing testes and 50 female hydra bearing eggs were cultured together in 100 ml of hydra medium. The males were removed weekly and replaced with new males bearing testes; 50–100 fertilized eggs, identified by the initiation of cleavage, were harvested. In most cases, the strands attaching the embryo to the parent were cut with a surgical knife and the detached egg was transferred to a petri dish (60 mm × 15 mm) containing hydra medium. In other cases, females carrying fertilized eggs were transferred to a fresh dish containing hydra medium, and the embryos were allowed to develop while still attached. Cultures of 20 embryos or 20 females bearing embryos were maintained at 15°C, and the medium was replaced daily. Embryos were cultured until they hatched, unless used for experiments at some point during embryogenesis.

Analysis of embryonic stages

Video microscopy. Progress through the stages of embryogenesis was monitored on live embryos in two ways. For the early stages of development, embryos from the fertilized egg to the early cuticle stages were continuously monitored with a time-lapse video recorder (JVC BR-9000U) mounted on a dissecting microscope (Olympus SZH) and attached to a Sony Trinitron color monitor (model KX-2501). One frame per second was recorded over 3 days. Second, the entire process of embryogenesis, from fertilized egg to hatching, was monitored with an optical disc recorder (Panasonic) attached to a Wild dissecting microscope and a Sony color monitor. One frame per 15 seconds was recorded over 30 days. The resultant time-lapse films were viewed and analyzed at playback speeds 10-100 times normal speed.

Histology. At various stages from early cleavage to hatchlings, embryos or young animals were fixed and sectioned to examine their histological structure. The embryos were fixed in Lavdowsky's fixative (ethanol: formalin: acetic acid: water in the ratios of 50:10:4:40), either for 60 min at room temperature or, for embryos with a cuticle, overnight at 4°C. Embryos at the last stage—bilayer formation, which occurs 2 days before hatching—and young hatchlings were relaxed in 2% urethane in hydra medium for 30 s prior to fixation. Fixed embryos and hatchlings were dehydrated through an ascending alcohol series (25%–100% ethanol), rinsed for 20 min in a 1:1 mixture of 100% ethanol and tertiary butyl alcohol, incubated overnight in tertiary butyl alcohol, and then infiltrated and embedded in Paraplast Plus paraffin (Fisher Scientific). Serial sections (10 μm) of embryos and hatchlings were prepared, mounted on glass slides, and stained with Schiff's reagent, toluidine blue, or hematoxylin and eosin. The stained sections were observed and photographed with a Zeiss compound microscope.

Scanning electron microscopy. Relaxed hatchlings and

embryos at various stages were fixed for 90 min with glutaraldehyde (2.5% in 10 m*M* Millonig's phosphate buffer, pH 7.2), and then rinsed three times for 15 min each in 10 m*M* Millonig's buffer. While in buffer, some samples were cut transversely with a straightedged razor blade. Samples were postfixed for 60 min in 2% osmium tetroxide in 10 m*M* Millonig's buffer, and then rinsed three times in 10 m*M* Millonig's buffer. Subsequently, they were dehydrated through a graded series of ethanols to 100%, critical-point dried with CO_2, mounted on metal stubs, and sputter-coated with gold-palladium for 1 min in a Denton sputter-coater. Samples were examined and photographed with a scanning electron microscope (JEOL JSM T-300) operated at 25 kV.

Quantification of cell numbers and types. Individual embryos were submerged in 50 μl of maceration fluid (acetic acid:glycerol:water in the ratios of 1:1:26; David, 1973) and maintained at 5°C for 2–3 days. For eggs and embryos up to the cuticle stage, the cell suspension was gently agitated with a stream of air blown through a microcapillary pipette. The cells dispersed in this way were postfixed with 8% formaldehyde and transferred to a gelatin-coated slide. A piece of fishline coated with 1% Tween 80 was used to spread the cells evenly over a 1 × 1–2 cm^2 area of the slide. For later stages (post-cuticle formation), individual embryos were placed on a gelatin-coated slide in a drop of maceration fluid, and the cuticle was removed with sharpened forceps. This often caused some of the larger embryonic cells to be sheared as they were extruded through the opening in the cuticle layer. Nevertheless, the cells were postfixed with formaldehyde and spread on a gelatin-coated slide as described above. The cells from early and late stages were dried on a slide warmer (40°C) overnight.

To aid in distinguishing embryonic cells (or the nuclei released when cells were sheared) from the nurse cells that are present in eggs and embryos at all stages through to hatching, macerated cells were stained with a 2.5 μg/ml solution of DAPI (4,6 diamidino-2-phenylindole-2HCl; Accurate Scientific and Chemical Corp.) in 10 m*M* $MgCl_2$/McIlvaine buffer (18 m*M* citric acid, 164 m*M* Na_2PO_4, pH 7.0) for 30 min and rinsed in 10 m*M* $MgCl_2$/McIlvaine buffer. The cells were examined microscopically at 375× and classified using terminology established for cell types of adult hydra (David, 1973; Campbell and Bode, 1983). Classification criteria were based on nuclear morphology (examined with epifluorescence) for cells at early stages of embryogenesis and on both nuclear and cell morphology (viewed with phase optics) for cells at later stages.

Hydroxyurea treatment

To block cell division during gastrulation, blastulae were exposed to 10 m*M* hydroxyurea (HU) in hydra medium for 6–12 h at 15°C. Thereafter, the embryos were washed three times in hydra medium and allowed to develop in hydra medium until hatching. Some embryos (controls and treated) were fixed and processed for DAPI staining to determine if the treatment blocked cell division during the time of treatment. In detail, embryos were fixed in Lavdowsky's fixative for 60 min, rinsed in hydra medium, incubated for 5 min in DAPI, washed three times for 10 min each in phosphate buffered saline (PBS), mounted in glycerin:PBS (3:1), and examined with epifluorescence. The number of nuclei per embryo was scored. Some HU-treated embryos were processed for histology immediately after gastrulation as described above.

Immunocytochemical detection of mesoglea and interstitial cells

Hydra embryos (all stages of development) and hatchlings were examined for the presence of interstitial cells and mesoglea. The monoclonal antibody MG52 (kindly provided by Michael Sarras), which recognizes laminin (Sarras *et al.*, 1991), was used to detect the presence of mesoglea. CP4, a monoclonal antibody that recognizes cells of the interstitial cell lineage (Javois and Bode, unpubl. data), was used to identify cell types of this lineage. Indirect immunofluorescence was used for visualization.

The procedure used for immunocytochemistry was a modification of the one described by Dunne *et al.* (1985). Animals were fixed for 60 min in Lavdowsky's fixative, washed three times for 15 min each in PBT (PBS containing 0.25% Triton-X), and incubated overnight at 4°C in blocking serum (PBS containing 10% neonatal calf serum [Irvine Scientific] and 0.1% sodium azide). Thereafter, antibody diluted in PBS (1:100 dilution of CP4 ascites fluid or a 1:20 dilution of MG52 tissue culture supernatant) was added to the samples, and they were incubated overnight at 4°C. Subsequently, samples were washed two times for 10 min each in PBT followed by a 30-min rinse in PBT. Then, samples were incubated in a 1:50 dilution of FITC-conjugated goat anti-mouse Ig's (Boehringer-Mannheim) in blocking serum for 30 min in the dark at 22°C. Samples were washed three times for 10 min each in PBT and counterstained in 0.01% Evans blue in PBT for 10 min. Finally, animals were washed ten times for 2 min each in PBT, rinsed twice in PBS, and mounted on slides in 3:1 glycerin:PBS containing 0.5% *n*-propyl gallate, and examined with epifluorescence.

Results

Aspects of oogenesis

In hydra, an egg forms in the following manner (Honegger, 1981; Honegger *et al.*, 1989; Littlefield, 1994).

Stem cells located in the ectoderm, whose only differentiation products are oocytes and nurse cells, continuously enter the gamete differentiation pathway. Under the appropriate environmental conditions they complete traversal of the pathway, and the products, which have the morphology of large interstitial cells, accumulate in the ectoderm. One of these interstitial cells forms an oocyte that increases in mass by engulfing or fusing with large numbers of the remaining interstitial cells, referred to as nurse cells. As the oocyte grows in mass, it distends and eventually ruptures the ectoderm (Fig. 1). Thereafter, the ectoderm recedes around the edge of the egg to form the egg cup, a raised ring of tissue at the base of the egg (Fig. 2).

Once an egg is exposed to the medium, meiosis and fertilization occur. The egg nucleus, which is located at the distal end of the cell, the point furthest from the body column of the parent, undergoes meiosis I and II, resulting in the formation of three polar bodies (Honegger, 1981). Thereafter, in the female strain used here, the egg has to be fertilized within 2 h for normal embryogenesis to occur. After 2 h the addition of fresh sperm did not result in the initiation of cleavage divisions. Instead the egg began to swell and eventually disintegrated.

The nurse cells engulfed by the developing oocyte during oogenesis remain in the egg throughout embryogenesis as spherical, refractile cells with pycnotic nuclei (Zihler, 1972; Honegger *et al.*, 1989) (see Fig. 11). They are located within the cytoplasm of all cells until cuticle formation; thereafter, they persist in many of the epithelial cells until hatching. They occur in large numbers, ranging from 2000–9000 per embryo, with an average of 4500.

Stages of embryogenesis

1. Cleavage. Fertilization occurs at the distal end of the egg, which is the end farthest from the adult body column. The distal end is also the future head of the animal. This was established by marking the distal end with a vital dye and finding that the head was stained in the resulting hatchling. Cleavage in hydra embryos is holoblastic and unipolar; that is, the cleavage furrow progresses inward from one side of each cell. The first meridional division is initiated at the distal end of the embryo and moves in a distal-proximal direction perpendicular to the axis of the parent (Figs. 3 and 4).

The second cleavage division, which begins shortly before the first is complete, also occurs in a distal-proximal direction and is perpendicular to the first one. These two divisions result in four equal-sized blastomeres (Fig. 5). As the first cleavage furrow bisects the egg, small microvilli, or filopodia, form in the region immediately behind the leading edge of the furrow (Fig. 4). These microvilli, which also occur in the next cleavage divisions, appear to hold the forming blastomeres together. Occasionally the blastomeres separate at the two-cell stage, or less frequently at the four-cell stage, resulting in the development of two or four embryos in a single egg cup.

The third division is equatorial, starting at the side of the embryo closest to the head of the adult. The furrow occurs somewhat closer to the distal end of the embryo, resulting in two tiers of unequal-sized blastomeres (Fig. 6). Because the furrow starts at one end, the division of the four blastomeres is asynchronous. Thereafter, the cleavage divisions are very irregular. They occur asynchronously and the division planes are often oblique, yielding unequal-sized blastomeres. Cells near the distal end are generally smaller than those at the proximal end (Fig. 7).

By the fourth to fifth cleavage division, a cavity begins to form in the interior of the cell mass (Fig. 11), while the surface remains uneven (Fig. 8). Over the next 2 h the cavity enlarges to its final size. The shape of the embryo smooths out into a spherical shell (Fig. 9), which is organized in a single cell layer surrounding the blastocoel (Fig. 10). At this point, the embryo has reached the blastula stage and consists of 64–128 cells (ave. = 76 ± 47 cells, Table I). Each cell is filled with about 40 nurse cells (Fig. 11). Since each cell division lasts about 60 min, the blastula stage is reached within 6 to 8 h of fertilization.

2. Gastrulation. Gastrulation occurs by ingression. Shortly after the blastula is formed, individual cells begin elongating and extending their basal surfaces into the blastocoel (Figs. 15 and 16). Simultaneously, the lateral contacts with neighboring cells are reduced and eventually severed, resulting in these blastomeres moving into the blastocoel. The process of ingression occurs in about 2 to 4 h; when it is completed, the embryo consists of an outer layer of columnar cells and a central mass of unorganized spherical cells (Figs. 12, 17, 18). There are nurse cells in all blastomeres, although many more in the cells of the inner cell mass (Fig. 12). No cavity remains, and the embryo is somewhat compacted.

Ingression begins at the distal end and proceeds in a wave traveling across the embryo in a proximal direction. It is accompanied by a transient inward distortion of the surface of the blastula as cells ingress. This distortion apparently involves considerable force—an embryo detached from its parent will actually roll over the surface of the culture dish as ingression takes place.

During gastrulation, the number of cells increases roughly fourfold, from 76 ± 47 to 315 ± 200 (Table I). The fact that cell division occurs while cells are accumulating in the blastocoel suggests delamination as an alternative explanation for generating cells in the cavity. If

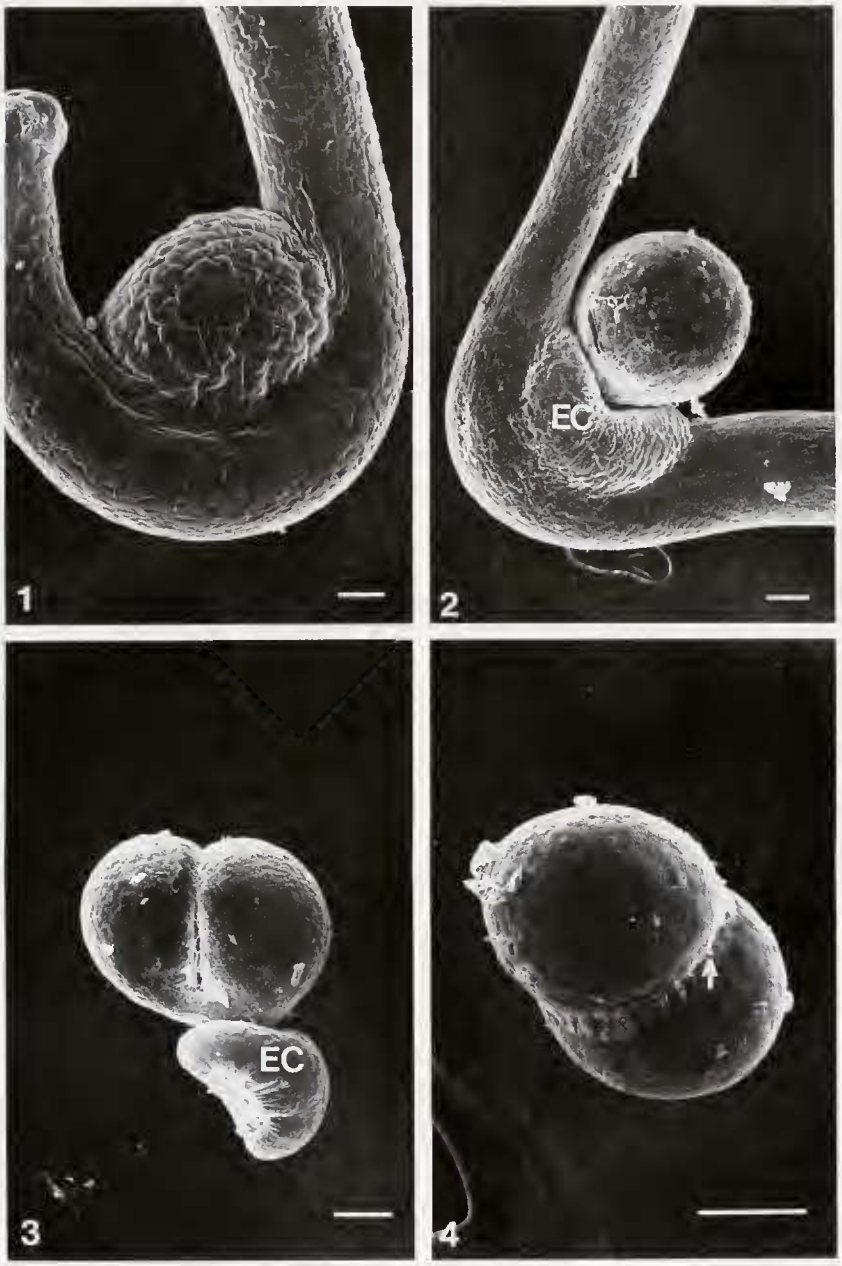

Figure 1. Hydra forming an egg. As the egg increases in size, it distends the overlying ectoderm of the parent. The ectoderm has not yet ruptured. Bar = 100 μm.

Figure 2. Hydra egg that has broken through the ectoderm. This developing egg is loosely attached to the parent at the egg cup (EC). Bar = 100 μm.

Figure 3. Hydra egg undergoing first cleavage. Note the distinctive heart shape created by unilateral furrowing. EC, egg cup. Bar = 100 μm.

Figure 4. Two-cell stage. Microvilli (arrow) form in the region of the cleavage furrow and may help hold the blastomeres together. Bar = 100 μm.

the plane of cell division were parallel to the surface of the embryo, cell division would result in one daughter remaining at the surface while the other would be in the interior. To distinguish between delamination and ingression, embryos were treated with hydroxyurea (HU) for 6 h covering the period of gastrulation. HU blocks cell division in hydra (Bode, 1983). In HU-treated embryos, gastrulation, the subsequent development of the embryo, and the resulting hatchlings were all normal. The only differences were two. One was that the cells of

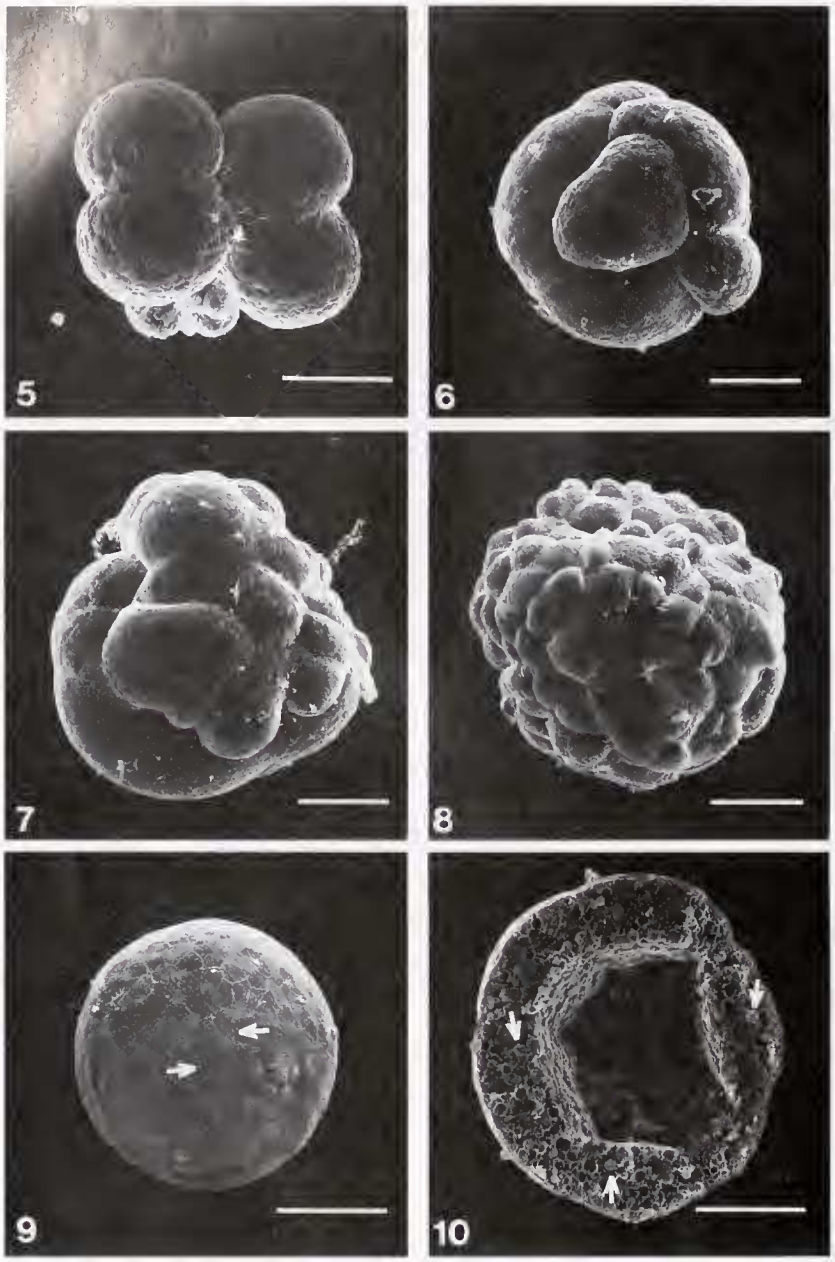

Figure 5. Four-cell embryo. Bar = 100 μm.

Figure 6. Forming eight-cell embryo of hydra showing two unequal-sized tiers of cells. Bar = 100 μm.

Figure 7. Mid-cleavage embryo. Note the unequal size of the blastomeres. Cells at the distal end are smaller than those at the proximal end. Bar = 100 μm.

Figure 8. Late cleavage stage. The cells are more uniform in size and resemble a morula. Bar = 100 μm.

Figure 9. Coeloblastula. Note the microvilli (arrows) that delineate the margins of the cells. Bar = 100 μm.

Figure 10. Cross-section of the coeloblastula. Note the single layer of cells surrounding the blastocoel. These cells contain many nurse cells (arrows). Bar = 100 μm.

ge[illegible] the treated animals were somewhat larger than in [illegible] (compare Figs. 12 and 13), as would be expected [illegible] vision had been interrupted. The other was that the [illegible] of cells did not increase during gastrulation in the treated animals. Thus, ingression of cells, not delamination, is the most likely explanation for the origin of the interior cells.

Gastrulation is complete by 8 to 12 h postfertilization,

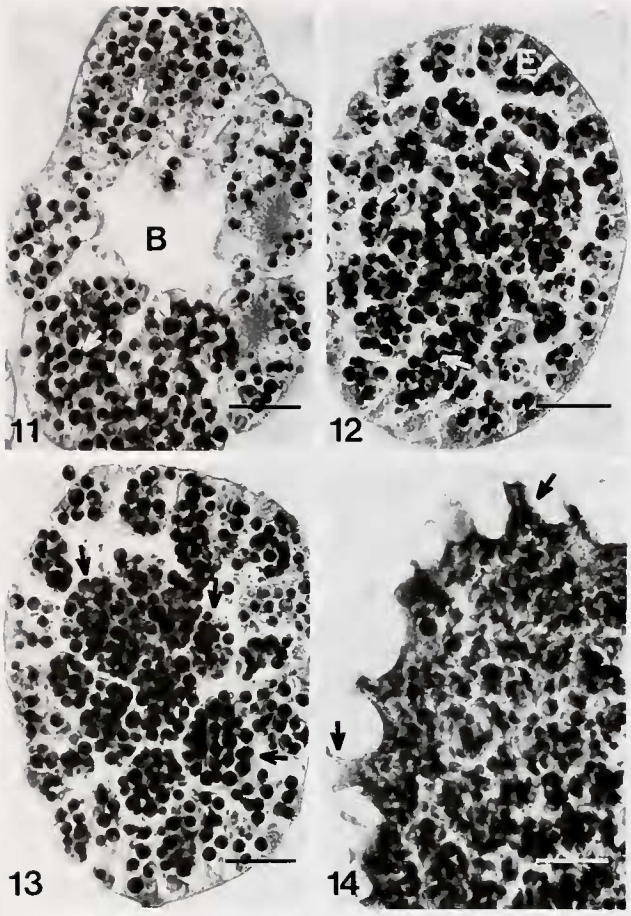

Figure 11. Histological section of the morula. Note the forming blastocoel (B) and the darkly stained nurse cells (arrows) within the blastomeres. Bar = 100 μm.

Figure 12. Gastrula of control embryo. A central mass of cells filled with darkly stained nurse cells (arrows) is surrounded by an outer epithelial layer (E). Bar = 100 μm.

Figure 13. Gastrula of hydroxyurea-treated embryo. Note the large cells (arrows) filled with nurse cells. Bar = 100 μm.

Figure 14. Cuticle deposition in a control embryo. Cells of the outer layer exhibit filopodia (arrows) extended into the outside medium. Cuticle material is deposited around the filopodia, producing spines. Bar = 100 μm.

or about 2 to 4 hours after the blastula is formed. Thereafter, little activity is visible for the next 10–12 h.

3. Cuticle formation. The next stage involves the formation of the cuticle, a thick protective outer layer that is also commonly referred to as the embryotheca. About 20 h after fertilization, the cells of the outer layer begin to extend filopodia into the surrounding medium (Fig. 14). Cuticular material is deposited in layers around the filopodia and on the apical surfaces of the cells (Fig. 19). Three hours later the material has built up to the point that spines are visible. Over the next 18–24 h, material is continually deposited, producing a multilayered structure over the surface of the embryo (Figs. 20 and 21) with ornate spines 25–50 μm long (Fig. 22) forming where filopodia were located. Towards the end of this period, the filopodia are retracted and the resulting channels filled with cuticular material. The process is complete 40–48 h postfertilization.

Cuticle deposition commonly starts on the proximal side in the egg cup and slowly progresses around the embryo in a distal direction. In general, the final distribution of material is somewhat asymmetric, with the cuticle being thickest (~60 μm) at the proximal end and thinnest (~45 μm) at the distal end. Once this process is complete, a second, very thin, membranous layer is deposited by the outer cell layer beneath the cuticle (Fig. 24). Thereafter, the embryo detaches from the parent, although detachment occurs occasionally at any point from the two-cell stage onward. The time of detachment has no bearing on the progress of embryogenesis.

4. Cuticle stage. Unlike the early and late stages of embryogenesis, the middle stage is of undefined length, ranging in the laboratory from 2–24 weeks. It is thought to correspond to an overwintering period of the embryo in nature. Two important events occur during this stage: the cells of the outer layer acquire characteristics of the epithelial cells of the adult ectoderm; and the cells of the interstitial cell lineage first appear.

Shortly after cuticle formation is complete, the outer layer is observed to consist primarily of smaller squamous epithelial cells, most of which are devoid of nurse cells (Figs. 23 and 24). In contrast, the interior cells are irregular in shape and all contain nurse cells (Fig. 25). A change in nuclear morphology is correlated with the appearance of these smaller epithelial cells. In macerates,

Table I

Increase in number of blastomeres and epithelial cells through embryogenesis

Stage	Time after fertilization	Number of epithelial cells[1] (± 1 SD)
Blastula	6–8 h	76 ± 47
Gastrula	8–12 h	315 ± 200
Cuticle formation	20 h	400 ± 200
Cuticle	40 h	370 ± 120
Cuticle	6 d	440 ± 160
Cuticle	11 d	420 ± 200
Bilayer[2]		3000 ± 100
Hatchling		2830 ± 1080

[1] Through 144 h, the cells are blastomeres and all are included. Thereafter, epithelial cells are counted, but cells of the interstitial cell lineage are not. Sample size = 5–13.

[2] Bilayer embryos are analyzed 2 days before hatching. Bilayer and hatchling data are from embryos with a diameter of 400 μm.

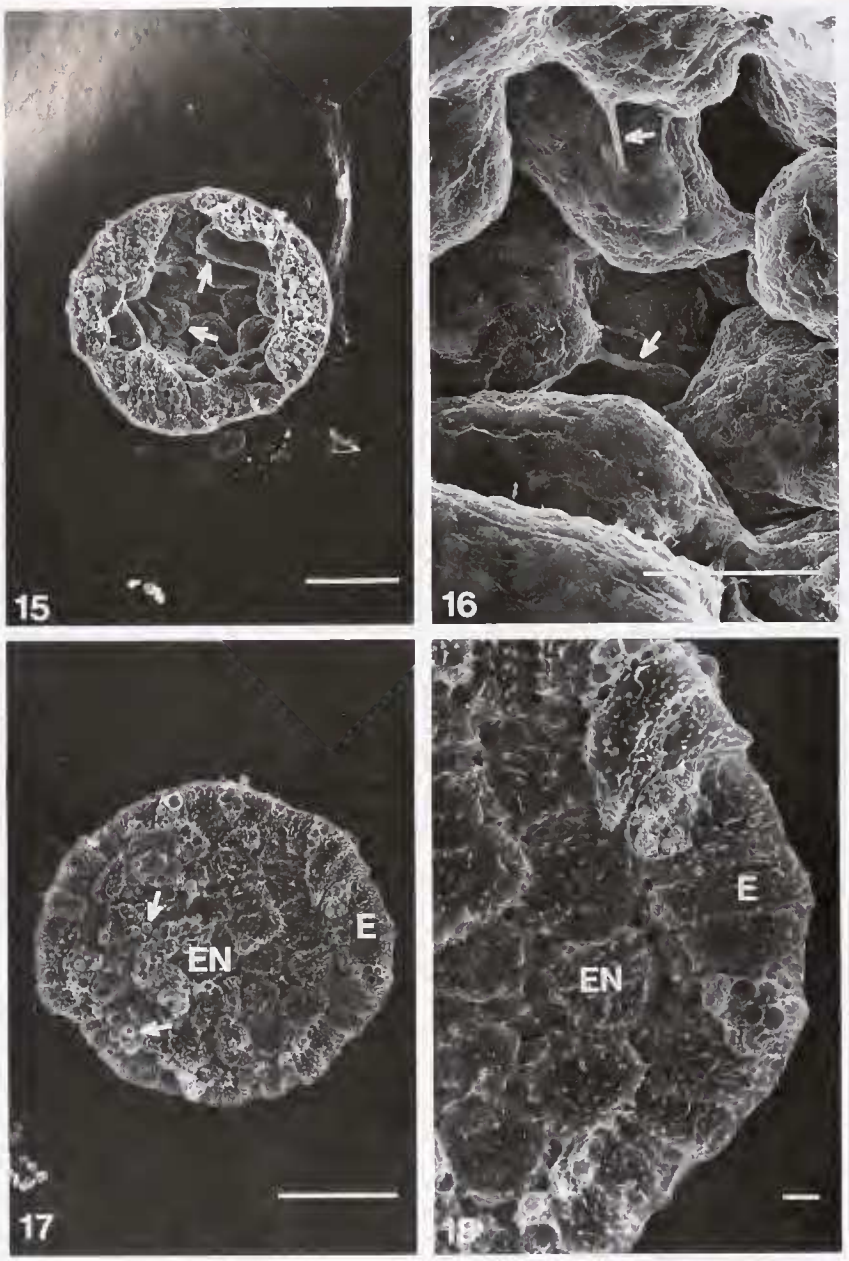

Figure 15. Cross-section of a hydra blastula showing cells (arrows) ingressing into the blastocoel from all sides. Bar = 100 μm.

Figure 16. Cells ingressing into the blastocoel during gastrulation (enlargement of Fig. 15). Individual cells extending filopodia (arrows) detach from their neighbors, move into the blastocoel, and fill it. Bar = 100 μm.

Figure 17. Cross-section of a control hydra gastrula. The outer layer of cells (E) will give rise to the epidermis of the adult polyp, while some of the cells of the inner mass (EN) will contribute to the gastrodermis of the adult. Nurse cells (arrows) are abundant in the inner layer of cells. Bar = 100 μm.

Figure 18. Cross-section of a control gastrula. Note the outer layer of columnar-shaped cells (E) that surround the central spherical cells (EN). A mesoglea is not detected between the outer and inner cells. Bar = 10 μm.

the nuclei of blastomeres of the gastrula and early cuticle stages are large and amorphous. By 6 days postfertilization and 4 days after cuticle formation is complete, some of the cells are much smaller and have nuclei that resemble those of the epithelial cells of adults. They have a prominent nucleolus and a clear cytoplasm. Thus, by

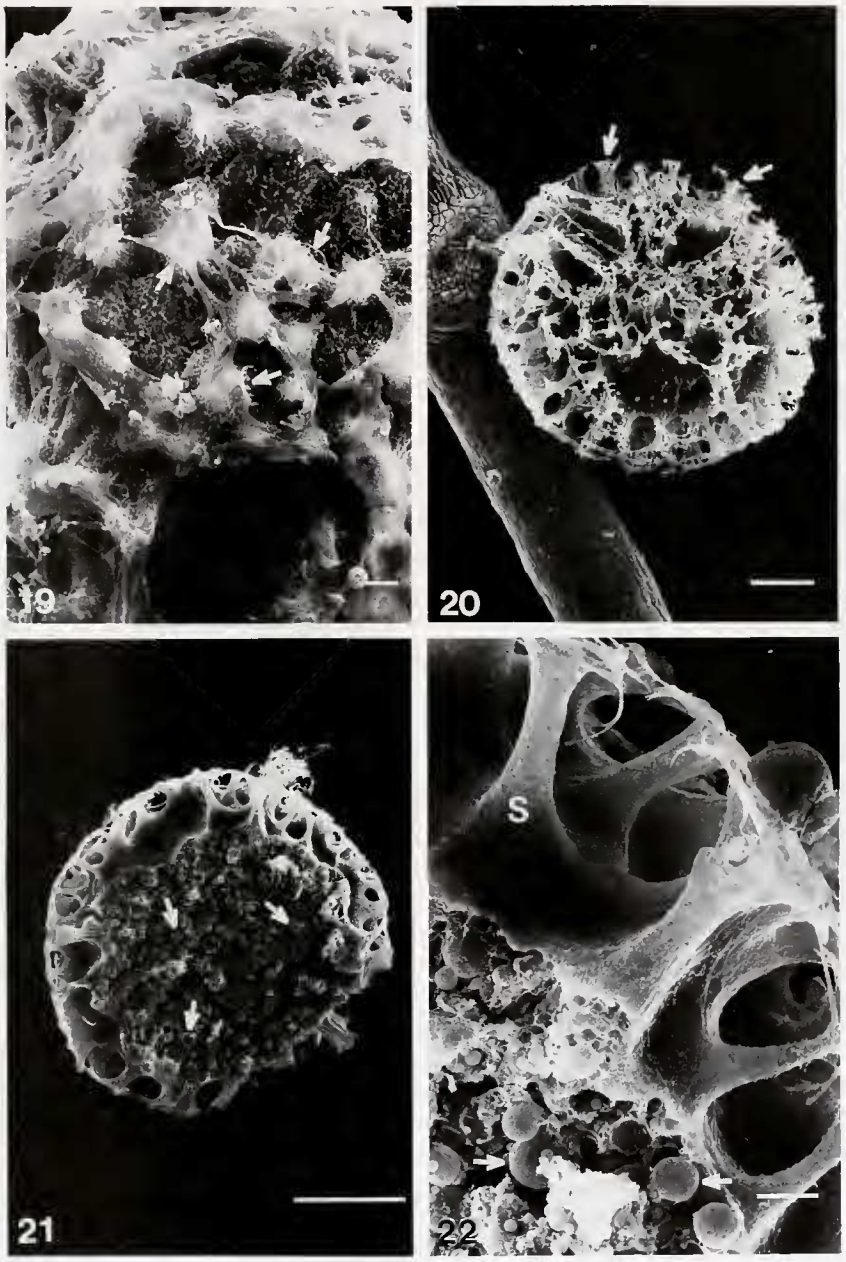

Figure 19. Early cuticle stage. Cuticular material (arrows) is deposited on the apical surfaces of the cells and around filopodia that project from the outer cells. Bar = 10 μm.

Figure 20. Hydra embryo with fully developed cuticle. The cuticle is adorned with thick spines (arrows). Bar = 100 μm.

Figure 21. Cross-section of a cuticulated embryo. A thick cuticle is tightly attached to an outer thin layer of squamous cells (not visible here). The interior of the embryo contains spherical cells rich in nurse cells (arrows). Bar = 100 μm.

Figure 22. Enlargement of cuticle spines (S) and nurse cells (arrows) of a cuticulated embryo. Bar = 10 μm.

this stage the outer layer becomes morphologically similar to the epithelium of the adult ectoderm.

The second event is the appearance of interstitial cells. All nonepithelial cells in hydra are part of the interstitial cell lineage. It consists of a population of interstitial cells, some of which are multipotent stem cells, and four classes of differentiation products: neurons, nematocytes, secretory cells, and gametes (Bode, 1996). During

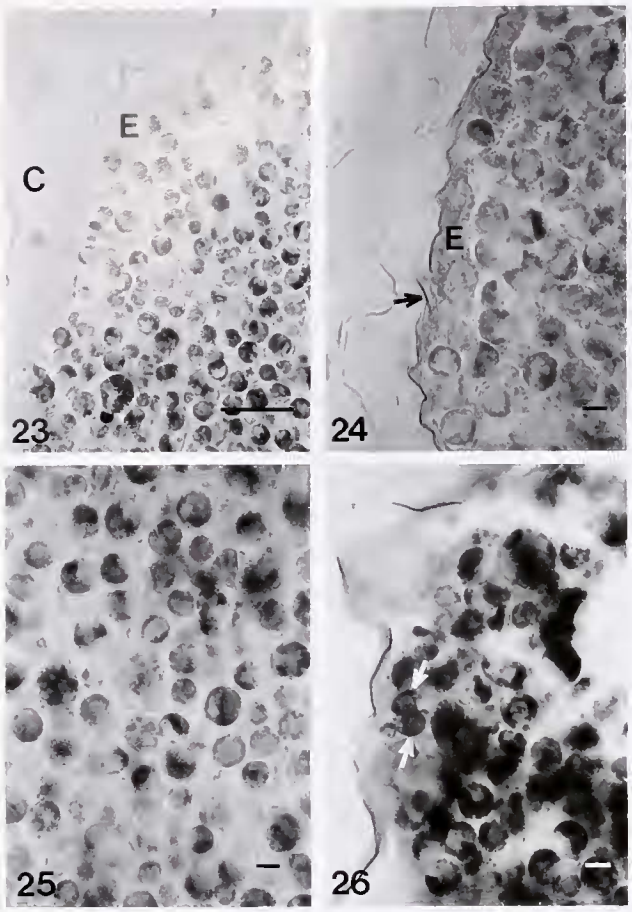

Figure 23. Early cuticle stage. The outer layer of lightly staining cells (E) is flattened against the cuticle (C). Nurse cells (darkly staining) fill the central mass of cells. Bar = 50 μm.

Figure 24. Mid-cuticle stage. Note the flattened squamous-like cells of the outer layer (E) and the inner membranous layer (arrow) of the cuticle. Bar = 10 μm.

Figure 25. Central mass of cells, containing nurse cells, of a cuticle stage embryo. Bar = 10 μm.

Figure 26. Young interstitial cells (arrows) among cells of the outer layer. Bar = 10 μm.

the first 6 days of embryogenesis, the morphology of the cells is either large with amorphous nuclei or epithelial in character. By 11 days postfertilization, however, another distinct change has occurred in a subpopulation of the cells. Much smaller cells with a morphology of the large and small interstitial cells of the adult have appeared (Fig. 26). Most of these cells are found among the epithelial cells of the thin outer layer (Fig. 26), and some are also observed in the inner mass of cells. As shown in Table II, most of these cells are large interstitial cells.

5 *Bilayer formation.* During this last stage of embryogenesis, two epithelial layers separated by a basement membrane are formed; these closely resemble the cell layers of the adult animal. Because the cuticle stage is of variable length, the timing of the onset of bilayer formation is not easy to predict. However, the fact that an embryo is in the final stage is signaled by a clear morphological change that occurs 2 days before hatching. In general, the embryo is opaque due to the refractility of the several thousand nurse cells in the cytoplasm of the embryonic cells. Two days before hatching the outer edge of the embryo becomes translucent. The thin outer layer of squamous cells devoid of nurse cells becomes thicker, consisting of columnar epithelial cells and large numbers of interstitial cells (Figs. 27 and 28). Between the 11-day postfertilization stage and the bilayer stage, the number of interstitial cells increases markedly (Table III). When sections are treated with the antibody CP4, which specifically recognizes large interstitial cells, the majority of stained cells appear in the outer layer (Fig. 29). In addition, the small interstitial cells (cell types derived from the large interstitial cells) appear in substantial numbers (Tables II and III). Small numbers of neurons and nests of nematoblasts are also observed. At this point, the layer closely resembles the ectoderm of the adult.

The formation of the ectoderm must precede the formation of the endoderm because the outer layer is clearly defined 2 days before hatching, while the cells in the interior remain in an unorganized mass (Fig. 27). Once the ectoderm has formed, some of the cells of the interior mass line up along the ectoderm and change in shape from spherical to columnar (Fig. 30). This alignment occurs simultaneously in different parts of the embryo, and the enlarging aligned regions of the developing endoderm fuse into a complete spherical layer. As this occurs, spaces appear among the cells within the interior cell mass (Figs. 28 and 30). These spaces enlarge and coalesce with time, thereby forming the gastric cavity. When these processes are complete, two layers have formed (Figs. 31 and 32). Most of the cells of the inner mass have become part of the endoderm, although some remain in the cavity and later appear to degenerate. The cells of the inner layer have the typical appearance of endodermal epithelial cells of the adult in that one or more cilia protrude from their surfaces into the gastric cavity (Fig. 32).

The last step is the formation of the mesoglea, the basement membrane that separates the two layers in the adult. Like the basement membranes found in higher metazoans, this structure is mainly composed of collagen IV, fibronectin, laminin, and heparan sulfate proteoglycan (Sarras *et al.*, 1991). Whole embryos at different stages of development were stained with the monoclonal antibody MG52, which recognizes the laminin component of mesoglea. Embryos at the blastula, gastrula, cuticle deposition, and early presumptive ectoderm stages did not stain with the antibody, indicating that the mesoglea had not yet been synthesized (data not shown). Embryos in which both layers had formed showed staining

Table II

Relative increase in the population sizes of the cell types of the interstitial cell lineage during embryogenesis

Stage[1]	Number of cells of a cell type/epithelial cell				
	Large interstitial cells	Small interstitial cells	Nematoblasts + Nematocytes	Neurons	Secretory cells
Cuticle (6 d pf)	—	—	—	—	—
Cuticle (11 d pf)	0.33	0.06	—	—	—
Bilayer	0.61	0.40	0.023	—	0.013
Hatchling (0–5 h)	0.52	0.58	0.13	0.025	0.058
Hatchling (2–20 h)	0.49	0.61	0.70	0.11	0.12
Adult[2]	0.64	0.70	1.32	0.25	0.27

[1] pf = postfertilization.
[2] Data from Bode *et al.*, 1973.

between the layers where the mesoglea normally forms (Fig. 33). The endodermal layer need not be complete for the appearance of the mesoglea, which begins to form shortly after the endodermal cells align on the ectoderm. With the formation of the mesoglea, the overall structure of the embryo is complete.

6. *Hatching.* Once the bilayer has formed, the embryo begins to pulsate rhythmically and continues to do so until hatching is complete. As the embryo pulsates, perforations and channels appear in the cuticle, forming a honeycomb pattern and suggesting that the structure is beginning to break down (Fig. 34). Within 24 h of the formation of the two epithelial layers, the cuticle cracks open on its thinnest side, the original distal side of the embryo where the head forms. To confirm that the crack was always on the distal side, embryos that had formed a cuticle were detached from the parent, embedded in soft agar with known orientation, and incubated in hydra medium. These embryos hatched in the agar, and in each case the cuticle opened at the distal end. Furthermore, the head end of the hatchling always came out first.

Once the cuticle opens, the spherical embryo continues its pulsatile contractile activity and begins to elongate and emerge from the cuticle (Fig. 35). For the next 2.5 h the periodic elongations and contractions continue, and the shape changes from a compact sphere to an elongate cylinder 550–700 μm in length. During the early stages of hatching, the embryo is still enveloped in the membrane that was laid down at the beginning of the cuticle stage, but as the embryo enlarges, the membrane ruptures. This results in increased activity of the hatchling as it frees itself of membrane and cuticle.

The apical end of the hatchling undergoes dramatic morphological changes during the final stages of hatching. Before the membrane ruptures, the apical end has the shape of a smooth dome (Fig. 35). Within as short a period as 15 min after rupture, the apical end narrows into a conical shape, and one to five tentacle primordia evaginate in a ring below the apical tip (Fig. 36). At this point, the head of the hatchling is morphologically complete and resembles that of a mature bud. The foot is also completely functional. Once free of the cuticle, the hatchling is able to stick to the surface of the petri dish. This ability is also found in adult hydra, and indicates the presence of functional mucous-producing cells in the foot region.

Other signs that the hatchling is essentially fully functional are apparent. The body column elongates and contracts, indicating that the muscle processes of the epithelial cells in each of the layers are fully developed. Further, the nerve net that coordinates the contractile activity must have formed. The hypostome, the dome above the ring of tentacles in the head, contains the mouth. In adults, treatment with glutathione results in opening of the mouth, mimicking a feeding response (Lenhoff, 1983). A freshly hatched hydra treated with glutathione opens its mouth, indicating that the mouth is fully formed and functional. When the hypostome is touched with a brine shrimp larva the mouth opens and the larva is ingested, and then digested. Because the tentacles are short upon hatching, capture of brine shrimp larvae is difficult. But within 2 days of hatching, after the tentacles have grown in length, the hatchling is fully capable of capturing shrimp larvae and feeding itself. Hence, the nematocytes necessary for the capture of the shrimp, the mucous cells of the head, which are inferred to be involved in ingestion, and the gland cells necessary for digestion have all formed. All of these activities indicate that the hatchling has a full complement of the somatic cell types found in an adult. As shown in Table II, the cell composition of a hatchling less than a day old is rapidly approaching that of an adult, which confirms the inference from the behavior of the young animal.

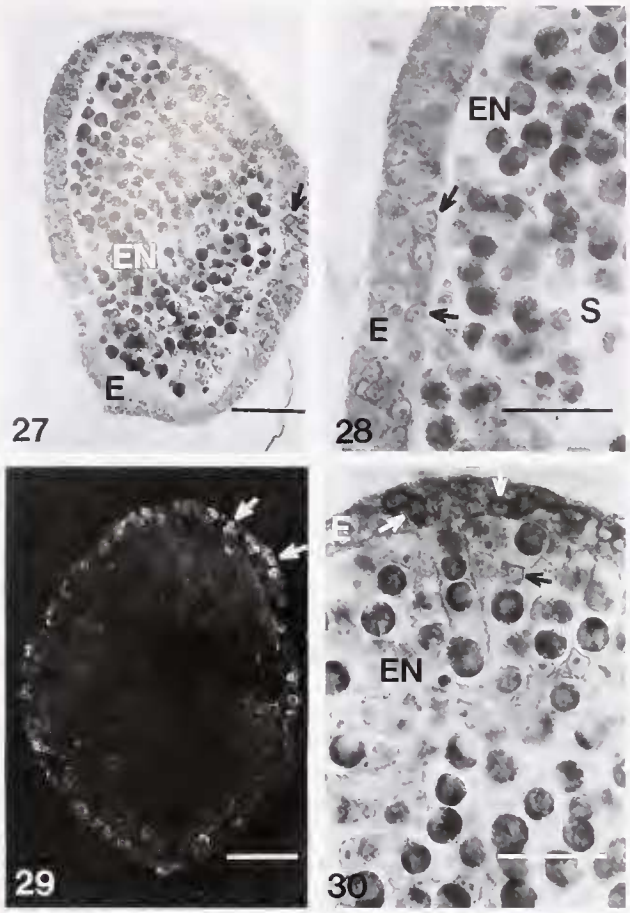

Figure 27. Early bilayer stage. An outer layer (E, future ectoderm) of columnar cells and interstitial cells (arrow) surround an unorganized mass of interior cells (EN, future endoderm). Bar = 100 μm.

Figure 28. Forming endoderm (EN) of a bilayer embryo. As the endodermal epithelial cells line up along the ectoderm (E), spaces (S) appear among the interior mass of cells. Note the interstitial cells (arrows) in the outer layer. Bar = 50 μm.

Figure 29. Bilayer stage stained with CP4 monoclonal antibody. A large number of labeled interstitial cells (arrows) are seen in the outer epithelial layer. Bar = 100 μm.

Figure 30. Bilayer stage. An outer ectoderm (E) and inner endoderm (EN) are complete. Interstitial cells (arrows) are found in both layers. Bar = 50 μm.

Discussion

Timing of embryological events

Because the body plan of hydra is simple, the events of embryogenesis are few when compared to the development of the body plans of more complex metazoans. The early events can be summarized as shown in Figures 37–39. After fertilization, the cleavage divisions lead to the formation of a single-layered blastula. Gastrulation is due to the ingression of cells from the blastula layer into the cavity until it is filled. Thereafter, a thick protective outer layer of cuticle and a thin inner membranous layer are laid down around the embryo (Fig. 40). During the prolonged cuticle stage, the major events are the conversion of the outer layer of blastomeres into a layer closely resembling the adult ectoderm and the appearance of the interstitial cell lineage (Figs. 41–43). In the final days before hatching, the endoderm, the mesoglea separating the two layers, and the gastric cavity form (Figs. 43 and 44).

When kept in the laboratory at a constant temperature of 15°C, embryos of the species used here complete development at any time from 2 weeks to 6 months. Yet, this is not an accurate reflection of the time required to undergo the events of embryogenesis. From fertilization through the completion of cuticle formation requires 40 to 48 h. The time from the appearance of the translucent stage, indicating a fairly complete ectoderm at the onset of the bilayer stage, until hatching is also about 48 h. The very large variable period occurs during the cuticle stage. In its normal freshwater habitats, the embryo is inactive during this period of embryogenesis.

There are indications that the actual time required for embryogenesis may be much shorter. Other authors (Kanaev, 1952) have noted that fertilized eggs can hatch in 13–14 days. We found that 5%–10% of the embryos hatched in about 2 weeks, but the percentage increased to 40%–50% if the embryos were subjected to electroporation at 5 days postfertilization (P. Bode, pers. obs.). These results suggest that, in addition to the 2 days at the beginning and 2 at the end of embryogenesis, the cuticle stage can be traversed in about 9 to 10 days. Because relatively few embryonic events occur during this stage compared to the beginning and end of embryogenesis, it is unclear whether this amount of time is obligatory.

The two epithelial cell lineages form sequentially

The development of both epithelial lineages involves relatively few steps. A hatchling derived from a large embryo (~400 μm in diameter) has about 3000 epithelial cells (see Table III). Most of the cell divisions giving rise to this population occur at the very beginning of embryogenesis. In the approximately 10 h from the fertilized egg through gastrulation, the cell number increases to about 300 cells, with each of the eight to nine cell divisions lasting about an hour. Thereafter, the rate of cell division slows down dramatically. In the next 10 h before cuticle formation begins, some of the cells undergo another division and raise the number to about 400 cells. Since the interstitial cells most likely arise by asymmetric cell divisions of blastomeres in the cuticle stage, one can consider the 400 blastomeres at the beginning of cuticle formation to be the direct precursors of the 3000 epithelial cells of the hatchling. This implies that these blastomeres, or later as epithelial cells, un-

Table III

Changes in cell composition during embryogenesis

Stage	Epithelial cells	Large interstitial cells	Small interstitial cells	Nematoblasts + Nematocytes	Neurons	Secretory cells
Blastula	76	—	—	—	—	
Gastrula	315	—	—	—	—	—
Cuticle formation	400	—	—	—	—	—
Cuticle (40 h)	370	—	—	—	—	—
Cuticle (6 d)	440	—	—	—	—	—
Cuticle (11 d)	420	140	25	—	—	—
Bilayer	3000	1830	1230	70	—	40
Hatchling (0–5 h)	2830	1470	1640	370	70	160
Hatchling (2–20 h)	2830	1390	1730	1980	310	340

Data for epithelial cells are from Table I. The number of cells of the interstitial cell lineages are calculated from the numbers of epithelial cells and the ratios of interstitial cell lineage cell type to epithelial cells from Table II.

dergo only another two to three divisions. And, if the minimum hatching time is about 13–14 days, about ten cell divisions occurred in the first day, and the remaining two to three occurred in the last 12–13 days.

The outer layer of blastomeres formed at gastrulation will give rise to the ectoderm. After ingression is complete, these cells still contain nurse cells, although they are fewer and mostly located near the basal portions of the cells (Fig. 39). In animals whose hatching time was not artificially reduced, a distinct change occurs by 6 days postfertilization. The outer layer next to the cuticle consists of a layer of squamous cells devoid of nurse cells (Fig. 41). In addition, the morphology of the nuclei of these cells changes from the amorphous nucleus of a blastomere to the clear nucleus, characterized by a prominent nucleolus, of an adult epithelial cell. This layer most likely arose from blastomeres of the outer layer undergoing a division parallel to the surface and giving rise to the squamous cells next to the outer cuticle and deeper, somewhat larger, cells that contain nurse cells.

The next distinct change in this layer occurs 2 days before hatching when the ectodermal layer becomes

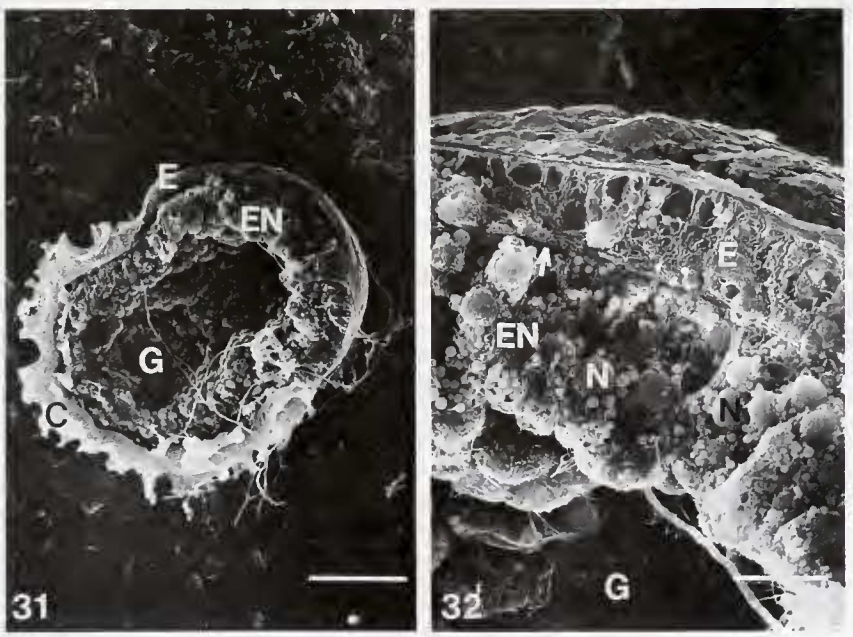

Figure 31. Cross-section of a bilayer stage embryo. Two distinct layers (E, EN) surrounding a gastric cavity (G) are visible. C, cuticle. Bar = 100 μm.

Figure 32. Bilayer stage embryo. A distinct ectoderm (E) and endoderm (EN) are separated by a mesoglea (arrow). Nurse cells (N) are found in the cells of the endoderm. G, gastric cavity. Bar = 10 μm.

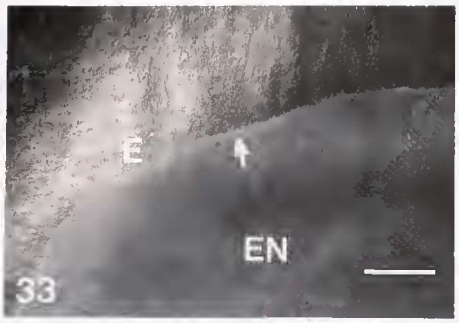

Figure 33. Mesoglea (arrow) of the bilayer stage stained with an anti-laminin monoclonal antibody, MG52. E, ectoderm; EN, endoderm. Bar = 50 μm.

translucent. The layer becomes two to three times thicker, with the ectodermal cells changing from squamous to columnar in shape. The increased width and the absence of the opaque nurse cells render the layer translucent. This change may occur primarily because of the rapid accumulation of cells of the interstitial cell lineage among the cells of the outer layer during the bilayer stage. Since the surface area of this layer is constant, the increasing mass of the interstitial cells may force the epithelial cells to undergo the observed shape change. At this point the outer layer is very similar to the adult ectoderm.

At the bilayer stage when the ectoderm is mostly complete, the definitive endoderm is nonexistent. The cells that will form the endoderm are still a disorganized mass in the interior of the embryo. Between the bilayer stage and hatching 2 days later, many of these cells begin to align themselves on the overlying ectoderm, changing in shape from roughly spherical to columnar (Figs. 43 and 44). This alignment occurs independently in several places, with the enlarging patches merging and fusing into a complete endodermal layer. These cells still contain large numbers of nurse cells and will do so for several days after hatching. The role of the nurse cells is unclear, although the most probable explanation is that they provide a source of nutrients.

The last step in the development of the two epithelial layers is the formation of the mesoglea between them (Figs. 43 and 44). This occurs after the alignment of the endodermal cells with the overlying ectodermal cells. Evidence in adult animals indicates that both layers are involved in the formation of the mesoglea (Epp *et al.*, 1986; Sarras *et al.*, 1993). Most likely the same process occurs here.

These events lead to the formation of two layers separated by a basement membrane. An unanswered question concerns the changes in these epithelia that will lead to formation of the head and foot. Shortly after hatching, tentacles emerge from the apical dome, and upon stimu-

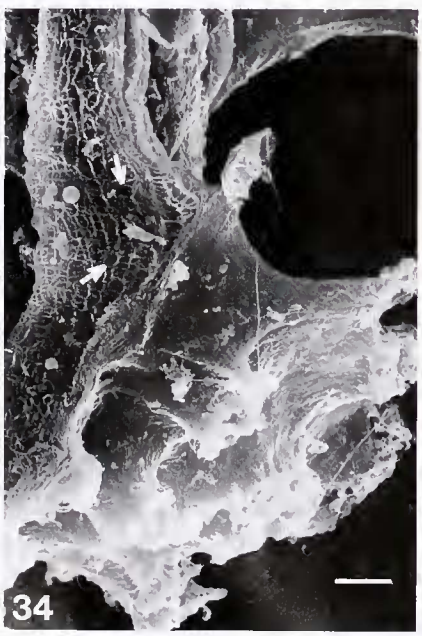

Figure 34. Degenerating cuticle just prior to hatching. Perforations and channels (arrows) appear in the cuticle, producing a honeycomb pattern. Bar = 10 μm.

Figure 35. Hydra hatchling emerging from the cuticle. Arrow indicates a dome-shaped anterior head. Bar = 100 μm.

Figure 36. Hydra hatchling with four tentacle bumps. Bar = 100 μm.

lation with glutathione, the mouth opens. In addition, once free of the cuticle, the hatchling sticks to the surface, indicating that it has a fully formed foot. When do the epithelia undergo the changes that set up the tissue for these morphogenetic and differentiation events?

Development of the interstitial cell lineage

All nonepithelial cells in hydra are part of the interstitial cell lineage. The formation of this lineage occurs in parallel with the development of the two epithelial layers. Large interstitial cells are rare or absent at 6 days postfertilization, but are present by 11 days (Tables II and III). Since large interstitial cells are considerably smaller than blastomeres, the simplest explanation is that some of the blastomeres undergo asymmetric cell divisions to generate the interstitial cells. Though such divisions were not observed directly here, Noda and Kanai (1980) reported them in another species of hydra, *Pelmatohydra robusta*, and Fennhoff (1980) described similar divisions in *Tubularia crocea*, a marine relative of hydra.

Where do these asymmetric cell divisions take place? Noda and Kanai (1980) placed them at the base of the outer layer, and Fennhoff (1980) localized them in the central mass of cells. In our study there is clear evidence of large interstitial cells in the thin outer layer where the epithelial cells are devoid of nurse cells. However, some cells in the interior mass of cells also have the morphology of interstitial cells. Results of the CP4 staining in bilayer stage embryos (Fig. 29) indicate that most of the interstitial cells are in the ectoderm, and some are in the

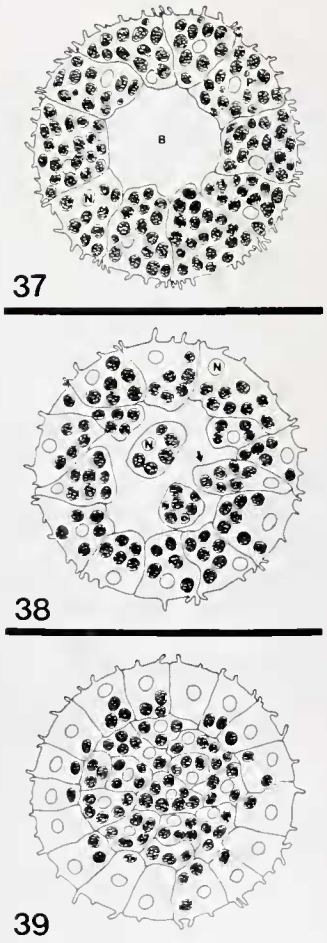

Figure 37. Coeloblastula. A single layer of cells filled with darkly colored nurse cells (P) surround a fluid-filled blastocoel (B). N, nucleus.

Figure 38. Cells ingressing (arrow) into the blastocoel during early gastrulation. As cells move to the interior they elongate, detach from their neighbors, and round up once in the filling blastocoel. N, nucleus.

Figure 39. Embryo at the completion of gastrulation. An outer layer of columnar cells has formed; within these cells the nurse cells are found at the basal tips. Spherical cells filled with nurse cells occupy the center of the embryo. A blastocoel is obliterated.

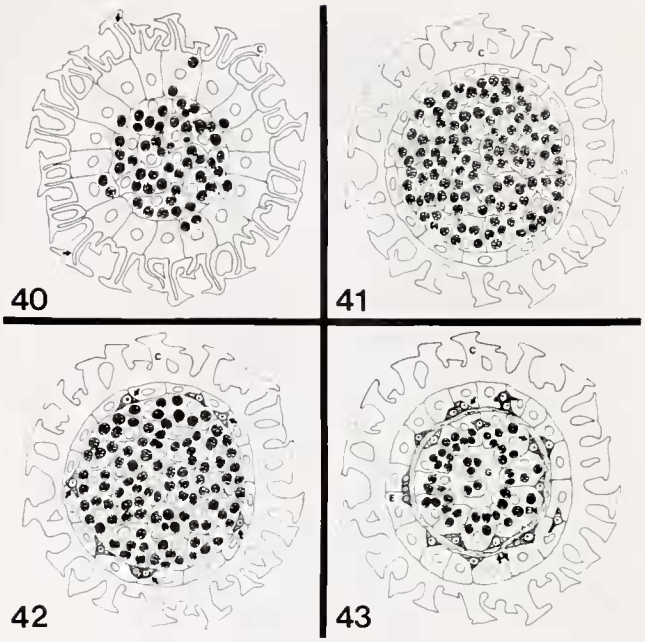

Figure 40. Early cuticle stage of hydra. The cells of the outer layer extend tiny filopodia (arrows) upon which cuticle material is deposited. Note that the darkly colored nurse cells have accumulated in the central cells. C, cuticle.

Figure 41. Embryo a few days after cuticle deposition is completed. Note that the outer layer of cells has flattened against the cuticle (C) to assume a squamous-like morphology. Nurse cells are virtually absent from the outer layer. Spherical cells containing numerous nurse cells fill the center of the embryos. The cuticle is thick and bears beautiful geometric spines. Filopodia of the outer cells have been retracted.

Figure 42. Mid-cuticle stage of hydra. Interstitial cells (arrows) arise in the outer layer. Cells of the outer layer have a flattened, squamous morphology. The inner cells are spherical and filled with nurse cells; no nurse cells remain in the ectoderm. C, cuticle.

Figure 43. Bilayer stage. The two layers, the ectoderm (E) and the endoderm (EN), are distinct and are separated by a mesoglea (double arrows). A forming gastric cavity (G) is present. Interstitial cells (single arrows) are found in both germ layers. C, cuticle.

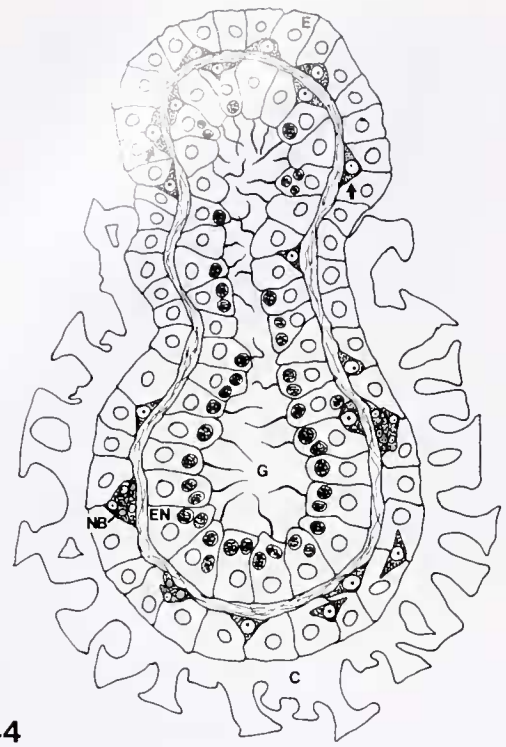

Figure 44. Emerging hatchling. As the cuticle (C) breaks open, the hatchling exits head-end first. The cells of the ectoderm (E) and endoderm (EN) expand, and the gastric cavity (G) increases in size. Interstitial cells (single arrows) are abundant in both layers, and clusters of nematoblasts (NB) (differentiating nematocytes) are found in the ectoderm.

endoderm. Since interstitial cells can migrate (Martin and Archer, 1986; Teragawa and Bode, 1990), it is quite plausible that they arise in both areas and that most eventually migrate into the ectoderm.

The development of the population of the interstitial cell lineage can be conveniently examined in three stages: the cuticle and bilayer stages and shortly after hatching. The large interstitial cells, which contain the multipotent stem cells (David and Murphy, 1977), arise during the cuticle stage, appearing between 6 and 11 days postfertilization. At 11 days the ratio of large interstitial cells to epithelial cells is 0.3: 1.0, and rises to 0.5: 1.0 by the time of hatching (Table II). In the adult, this ratio of these two ever-growing populations is maintained at 0.65: 1.0 (Bode *et al.*, 1973). These results imply that the population of large interstitial cells arises, grows, and approximates the adult ratio of large interstitial cells to epithelial cells during the cuticle stage. Whether the population of large interstitial cells reaches this size primarily by the division of a few interstitial cells that arise by asymmetric divisions, or whether most arise from asymmetric divisions is not known.

During the cuticle stage, most of the large interstitial cells are involved in proliferation. By the bilayer stage, however, a substantial fraction are differentiating, as measured in terms of the small interstitial cells, which are the dividing intermediates of the neuron and nematocyte pathways (David and Gierer, 1974). Shortly after hatching, fully differentiated neurons, nematocytes, and secretory cells appear; within a day there are substantial numbers of all three classes of these differentiation products. Since the time required for the three classes of differentiation products to traverse the differentiation pathways is equal to, or considerably longer than, the length of the bilayer stage, large interstitial cells must have begun entering the differentiation pathways late in the cuticle stage. Within a day after hatching, when the first wave of differentiation is complete, the ratios of the populations of these differentiation products to epithelial cells have reached half those found in adult animals (Table II).

In summary, the interstitial cell lineage develops in a straightforward manner. Large interstitial cells, perhaps all stem cells, appear during the cuticle stage and proliferate. Starting one-to-several days before the bilayer stage, these cells enter the three differentiation pathways in large numbers, resulting in the formation of numbers of neurons, nematocytes, and secretory cells within a day after hatching. Thus, this initial round of differentiation results in a cell composition that is close to that of the normal adult.

A mechanism for the formation of the gastric cavity and hatching

Unexplained so far is how the gastric cavity forms, and how an embryo emerges from the cuticle during hatching. Both events are probably part of a single process that is related to the osmotic behavior of the animal and the hydrostatic forces generated by this behavior.

There is a considerable difference in osmolarity between the external environment and the cytosol of the cells of an adult hydra (Lilly, 1955). Since the two environments are separated only by a plasma membrane, water is continually transported from the surrounding medium into, and through, the cells of the two epithelial layers into the gastric cavity. The increasing volume of the gastric cavity is periodically reduced as the animal contracts, thereby expelling fluid through the mouth.

Observations of developing aggregates indicate that both layers are necessary for this transport. When viable cells obtained by dissociating whole hydra are centrifuged into a pellet, or aggregate, the aggregate will develop into a normal animal (Gierer *et al.*, 1972). Initially, ectodermal epithelial cells migrate to the surface, forming an epithelium. Thereafter, endodermal epithelial cells align themselves on the ectoderm and form patches

of epithelium, which eventually coalesce into a complete endoderm. Internal spaces of fluid first form directly behind the patches of endodermal epithelium (Sawada, 1994), which suggests that the transport of water from the surrounding medium into the interior requires both the ectodermal and endodermal layers.

A similar process occurs during embryogenesis. During the bilayer stage, as the endodermal layer forms, fluid-filled spaces appear among the internal cells; these eventually coalesce into the gastric cavity. Because the size of the cavity becomes substantially larger than the volume of the space occupied by the interior cells at the beginning of the bilayer stage, it is unlikely that the cavity arises simply by the ordering of these cells into a layer. Instead, it is more likely that the formation of the gastric cavity and the subsequent hatching are due to the transport of water into the internal space. This could happen in the following manner.

By the bilayer stage, the cuticle has developed cracks. Because the cuticle is thinnest at the distal end where the head will form, it is likely that water seeps through the cuticle in significant quantities in this location first, and is transported into the interior of the embryo. That the gastric cavity is first visible as an elipse-shaped space near the distal end is consistent with this view. As the cuticle weakens, increasing amounts of water are transported into the gastric cavity from all over the surface of the embryo. This increases the hydrostatic pressure of the cavity, which in turn increases the pressure on the weakening cuticle. Eventually the cuticle cracks at the thinnest, and thus, weakest, point, which is at the distal end where the head is forming. With the embryonic surface now in direct contact with the surrounding medium, even more water is transported across the epithelial layers. Without the restraint of an intact cuticle at the distal end, the ever-increasing hydrostatic pressure in the gastric cavity distends the epithelial layers. Hence, the embryo slowly emerges through the crack in the cuticle like an inflating balloon, increasing in size as it emerges. Once liberated, the hatchling is at least twice the size it was just before hatching.

Another effect of water transport is taking place at the same time. Adult ectodermal epithelial cells have vacuoles that make up 90%–95% of the cell volume (Campbell, 1985). The size of these vacuoles is inversely correlated with the ion concentration of the surrounding medium (Trenkner *et al.*, 1973). Shortly before hatching, the ectodermal epithelial cells have no, or small, vacuoles. As water enters the late stage embryo during prehatching and hatching, the ectodermal cells begin to form vacuoles and increase in size. The resulting increase in the size of the ectoderm as a whole acts synergistically with the increasing volume of the gastric cavity to force the hatchling out of the cuticle.

Embryogenesis in hydra and deuterostomes shares several characteristics

The evolutionary distance is considerable between the diploblastic, radially symmetrical cnidaria and the triploblastic bilateral phyla of both deuterostomes and protostomes. Yet, in examining the evolutionary conservation of developmental processes and mechanisms, it is useful to compare the features of embryogenesis in hydra, or cnidaria in general, with those of other phyla. A tentative conclusion is that embryogenesis in hydra has a number of features that are characteristic of deuterostomes.

The cleavage pattern of hydra is radial for the first three divisions, becoming irregular thereafter. Both are characteristic of some vertebrates, such as amphibians. The resulting hydra blastula is a spherical shell consisting of a single layer of cells surrounding a blastocoel, as is found in sea urchins. And, as with many deuterostomes, hydra embryos are regulative during early cleavage stages. The two cells resulting from the initial cleavage sometimes separate, with each blastomere developing individually into a normal animal. Each of these embryos is half the size of the original egg. Occasionally, the cells resulting from the first two cleavage divisions separate to form four embryos with one-fourth the volume of the original egg. They too undergo normal embryogenesis and yield normal hatchlings.

In many deuterostomes, gastrulation occurs by involution or invagination of parts of the blastula. The same is true for some cnidarians, as many scyphozoans and anthozoans gastrulate by invagination (Hyman, 1940; Berrill, 1949; Chia and Spaulding, 1972). Other cnidarians use ingression for gastrulation; hydra clearly uses this process (Hyman, 1940). The endoderm and mesoderm of the chick embryo are also formed by ingression, as are the primary mesenchyme cells of the sea urchin embryo.

Another interesting feature is that, like vertebrates, hydra has several stem cell systems that arise during embryogenesis. Both the ectoderm and the endoderm of hydra are epithelial stem cell systems; they are similar to the epidermis and intestinal epithelium of vertebrates in that all consist of stem cells and differentiation products. Even more striking is the hydra interstitial cell system, which has many similarities to the hemopoietic system of the mouse. In both cases the cells of the tissue are migratory, and a multipotent stem cell gives rise to a number of differentiation products.

Finally, the cnidaria are usually considered to be diploblastic animals with an ectoderm and an endoderm, but no mesoderm. They may, in fact, have a primitive mesoderm. The interstitial cell lineage is a separate lineage even though it is physically intermingled with the

cells of the other two layers. Like many mesodermal tissues, this interstitial cell lineage is mesenchymal rather than epithelial in character. Moreover, in some cnidarians there are cells of the interstitial cell lineage in the mesoglea, suggesting the beginnings of a physical separation of a mesoderm "precursor" from the other two layers.

Conclusion

This study provides a clearer understanding of the sequence and timing of embryonic events in hydra, the changes in cell population sizes, and the development of the three cell lineages that constitute the adult animal. We are now in a better position to examine the expression pattern of a variety of genes that have been isolated in hydra, and thereby investigate the relationship among the patterning processes that govern embryogenesis, budding, and maintenance of the form of the adult body.

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